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**13. ABSTRACT (Maximum 200 Words)**
The androgen receptor (AR) is a ligand-regulated transcription factor that stimulates cell growth and differentiation in androgen-responsive tissues. The AR N-terminus contains two activation functions (AF-1a and AF-1b) that are necessary for maximal transcriptional enhancement by the receptor, however, the mechanisms and components regulating AR transcriptional activation are not fully understood. We sought to identify novel factors that interact with the AR N-terminus from an androgen-stimulated human prostate cancer cell library using a yeast two-hybrid approach. A 157-amino acid protein termed ART-27 was cloned and shown to interact predominantly with the $AR_{153-336}$, containing AF-1a and a part of AF-1b, localize to the nucleus and increase the transcriptional activity of AR when overexpressed in cultured mammalian cells. ART-27 also enhanced the transcriptional activation by $AR_{153-336}$ fused to LexA DNA binding domain, but not other AR N-terminal subdomains, suggesting that ART-27 exerts its effect via an interaction with a defined region of the AR N-terminus. ART-27 interacts with AR in nuclear extracts from LNCaP cells in a ligand-independent fashion. Interestingly, velocity gradient sedimentation of HeLa nuclear extracts suggests that native ART-27 is part of a multiprotein complex. Thus, ART-27 is a novel cofactor that interacts with the AR N-terminus.

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**INTRODUCTION**

Our overall objective is to elucidate the molecular mechanisms of transcriptional regulation by the androgen receptor (AR) in prostate cancer. AR is a hormone-dependent transcription factor involved in the regulation of both normal and malignant prostate cell growth by controlling target genes and signaling pathways involved in cellular proliferation. AR-mediated transcriptional activation is controlled by associations with as yet unidentified cofactors involved in transcription regulation, termed coactivators and corepressors. We have identified and characterized a 157-amino acid protein termed ART-27 one such cofactor, termed ART-27, which interacts predominantly with the $AR_{153-336}$, containing AF-1a and a part of AF-1b, localize to the nucleus and increase the transcriptional activity of AR when overexpressed in cultured mammalian cells. Understanding how ART-27 governs AR transcriptional activation in prostate cancer can be exploited in the development of new therapies for AR-dependent prostate cancer.

**BODY**
Statement of Work (Year 1).
Task1. Identification and Characterization of AR AF-1 Interacting Proteins

a.  Cloning and characterization of the AR AF-1 Interacting Protein, ART-27
b.  Expression analysis of ART-27
b.  Expression of ART-27
c.  Specificity of ART-27 interaction


Cloning and characterization of ART-27  (Task 1a)
To identify proteins that interact with AR N-terminus, we elected to screen for interacting proteins using a yeast two-hybrid system. Several positive clones were identified; one of which was termed Androgen Receptor Trapped clone 27 (ART-27). The clone from the yeast two-hybrid assay corresponded to the full-length cDNA and contained an insert of ~900 bp, in which the largest open reading frame (ORF) encoded a protein of 157 amino acids with an estimated MW of 18 kDa. A BLAST search of the Genebank database revealed that ART-27 is located on the X chromosome (Xp11.23-11.22) and is identical to a recently identified ORF of unknown function, termed ubiquitously expressed transcript (UXT; Accession number AF092737), prevalent in tumor tissues (Schroer et al., 1999). The

ART-27 protein contains potential phosphorylation sites for protein kinase A, protein kinase C and casein kinase II, but no other obvious motifs as determined by PROSITE (Fig. 1B) (Hofmann et al., 1999). Secondary structure prediction algorithms suggest that ART-27 is composed of multiple, successive alpha helices (Chou and Fasman, 1978; Garnier et al., 1978). ART-27 also appears to be conserved throughout evolution, with model organisms *Mus musculus* (AF092738), *Arabidopsis thaliana* (AC006535), *Drosophila melanogaster* (AE003412) and *Caenorhabditis elegans* (U40934), displaying 79%, 55%, 49% and 26% percent similarity, respectively, with human ART-27. Interestingly, ART-27 showed no significant homology to *Saccharomyces cerevisiae* proteins, suggesting that ART-27 first arose in metazoans, as did nuclear receptors (Amero et al., 1992; Owen and Zelent, 2000).

Expression pattern of ART-27 (Task 1 b)

A polyclonal antibody was made against the ART-27 C-terminus. Immunoblot analysis of HeLa and PC-3 cell nuclear extracts show that the antibody recognizes a single endogenous protein with an estimated molecular mass of ~18 kDa that migrates at the same apparent molecular weight as the cloned ART-27 expressed in COS-1 cells (Fig. 1B, compare lanes 1, 4 and 6), confirming that the ORF predicted from sequence analysis was indeed ART-27. No difference in ART-27 expression was observed between untreated and phorbol ester (TPA)- treated HeLa nuclear extracts (Fig. 1B, compare lanes 1 and 2), suggesting that activation of protein kinase C does not affect ART-27 expression or electrophoretic mobility indicative of phosphorylation. However, a slight increase in ART-27 expression was observed in serum-stimulated HeLa nuclear extracts as compared to untreated control cells (Fig. 1B, compare lanes 1 and 3), suggesting that ART-27 expression may be regulated by extracellular signals or through the induction of cellular proliferation.

Northern hybridization analysis was carried out to determine the expression pattern of ART-27 mRNA in human tissues. A single transcript of ~0.9 kb is present at variable levels in the human tissues examined, with the highest levels in the heart, skeletal muscle, kidney, liver, adrenal gland, lymph node, prostate, thyroid, and the lowest levels in bladder and uterus. We also performed Northern blot analysis on mRNA isolated from androgen-independent (PC-3) and androgen-dependent (LNCaP) prostate cancer cells, either untreated or treated for 72 h with the synthetic androgen R1881 at the indicated concentrations

(Fig. 1C). ART-27 steady state mRNA expression is slightly higher in PC-3 relative to LNCaP cells and is weakly induced in LNCaP cells in response to androgen.

ART interaction specificity (Task 1b)

To analyze the specificity of AR:ART-27 interaction, we examined the capacity of ART-27 to associate with a panel of transcriptional regulatory proteins in the yeast two-hybrid system including the AR C-terminal ligand binding domain (LBD), the AF-1 region of the glucocorticoid receptor (GR), the estrogen receptor alpha (ER α, the steroid receptor coactivator-1 (SRC-1), the TBP-associated factor 130 (TAF$_{II}$130), the Sp1 (A and B domains), the cyclic AMP response element binding protein (CREB) and VP16. As shown in Figure 2, ART-27 interacts the AR N-terminus and weakly the GR N-terminus, as well as with ER_α Sp1 and TAF$_{II}$130 (rank order of interaction: AR N-terminus $_{18-500}$> TAF$_{II}$130>Sp1B>ERα=Sp1A>GR N-terminus, but not with SRC-1, CREB, VP16 or the AR or GR LBD in either the absence or presence of hormone. Proteins that did not interact with ART-27 have been shown to interact with other factors, suggesting that the lack of interaction with ART-27 is genuine. For example, SRC-1 has been shown to interact with ERα, whereas the AR and GR LBDs associate with GRIP-1 and CREB interacts with TAF$_{II}$130 in the yeast two-hybrid system (Hong et al., 1999; Saluja et al., 1998; Shibata et al., 1997). Thus, our results indicate that ART-27 interacts not only with the AR N-terminus, but also with at least two other SRs, and with certain other transcriptional regulators including TAF$_{II}$130.

ART-27 is involved in androgen receptor transcriptional activation (Task 2)

A transient transfection assay was used to examine the role of ART-27 in the regulation of AR transcription activation. AR-deficient HeLa cells were transfected with a constant amount of full length AR and increasing concentrations of an expression vector encoding a HA-ART-27 along with an AR-responsive luciferase reporter gene. As shown in Figure 3A, the hormone-induced AR transcriptional activation was increased in a dose-dependent manner by overexpressed ART-27. This effect was AR-dependent, since in the absence of AR, ART-27 did not influence reporter gene activity (Fig. 3A). This enhanced transcriptional activity did not result from increased AR protein production, as AR levels were not affected by ART-27

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coexpression (our unpublished results). The effect of ART-27 on AR was not restricted to a single cell type, since overexpression of ART-27 in PC-3 and COS-1 cells also increased AR transcriptional activity resulted in a dose-dependent (our unpublished results). Thus, ART-27 can act as a positive regulator of AR transcriptional activity in mammalian cells.

The ability of ART-27 to affect transactivation by other members of the SR family- GR, ERα, ERβ, and the thyroid hormone receptor β-1 (TRβ-1)- was tested using transient transfection assays. Our results indicate that ART-27 increased the transcriptional activity of all four receptors in a dose-dependent manner (Fig. 3B-D). We next tested the effect of ART-27 on VP16-dependent transactivation. Recall that VP16 did not interact with ART-27 in the yeast two-hybrid assay. Consistent with this lack of interaction, ART-27 expression had no effect on GAL4-VP-16 activity from a reporter plasmid containing 5 Gal4 binding sites upstream of the E1b promoter (Fig. 3E). Together, these results suggest that ART-27 increases transactivation by steroid and thyroid hormone receptors.

We also examined the ability of ART-27 to activate transcription when artificially recruited to promoters in mammalian cells. Our results indicate that recruitment of ART-27 to a promoter by fusing it to either the Gal4 (our unpublished results) or LexA (Fig. 3F) DNA-binding domain fails to activate transcription, even though the proteins were expressed (our unpublished results). In fact, transcriptional activation of the LexA-ART-27 derivative was slightly reduced relative to LexA, suggesting that ART-27 may interact with and sequester factor(s) responsible for "basal" promoter activity. Thus, ART-27 lacks an intrinsic transactivation function. This may suggest that ART-27 it is unable to overcome a rate-limiting step in transcription when artificially recruited to a promoter or, alternatively that ART-27 is only one target of the AR and that the receptor requires multiple targets to recruit mammalian Pol II and initiate transcription.

## Enhanced AR-dependent transcriptional activation by ART-27 is mediated through a distinct receptor N-terminal domain

Because ART-27 interacts strongest with the AR subdomain spanning amino acids 153-336, we expect that it would affect the transcriptional activation potential of this AR subdomain. To determine if ART-27 could affect the function of the different AR subdomains, AR N-terminal derivatives containing amino acids 18-156, 153-336, 336-500, and 18-500 were expressed as fusion proteins with the

LexA DNA binding domain. HeLa cells were transfected with the LexA-AR N-terminal derivatives, along with a LexA-responsive luciferase reporter gene in the presence and absence of ART-27. In the absence of ART-27 coexpression, all four subdomains of the AR N-terminus are capable of activating transcription of the LexA-luciferase reporter gene to varying degrees (Fig. 4A). Importantly, overexpression of ART-27 enhances the transcriptional activity of two AR derivatives containing the ART-27 interaction region, LexA-AR$_{153-336}$ and Lex-AR$_{18-500}$, but not that of the derivatives lacking this region, LexA-AR$_{18-156}$ and LexA-AR$_{336-500}$. In fact, transcriptional activation of the LexA-AR$_{336-500}$ derivative was slightly reduced by ART-27 overexpression, suggesting that ART-27 may interact with and sequester a factor responsible for AR transactivation via the 336-500 subdomain. Immunoblotting with an antibody against the LexA moiety common to all derivatives indicate that expression of these chimeras is unaffected by coexpression of ART-27 in HeLa cells (Fig. 4B). These results suggest that the enhancement of AR transcriptional activation by ART-27 is mediated via the ART-27 interacting region.

## ART-27 is part of a high molecular weight complex

Several transcriptional regulatory cofactors have been identified as components of multiprotein complexes. We therefore examined whether ART-27 is part of a higher-order species using velocity gradient sedimentation. HeLa cell nuclear extracts were applied onto 15% to 35% glycerol gradients, either containing or lacking 2.4 M urea. Following centrifugation, fractions were collected and analyzed by immunoblotting with antibodies specific for ART-27 or TBP. Previous work has shown that TAFs and TBP exist in a complex, which can be dissociated with urea (Tanese et al., 1991). It has been shown that the larger, faster sedimenting species corresponds to TBP and TAFs binding together as TFIID, whereas the smaller, slower migrating peak represents additional non-TFIID TBP complexes (Tanese et al., 1991). As expected, under native conditions, TBP sedimented at the bottom, in the high-density region of the gradient in two discrete peaks. Under native conditions, a majority of ART-27 also migrated within the high-density region of the gradient (estimated range of molecular mass between 240 and 700 kDa) (Fig. 5). In the presence of 2.4 M urea, the sedimentation patterns of TBP and ART-27 were shifted to the top of the gradient (Fig. 5). The change in mobility of TBP is in agreement

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with previous results demonstrating the sedimentation profile of dissociated TFIID (Tanese et al., 1991). These results are consistent with the idea that ART-27 is part of a high molecular weight complex, the components of which have yet to be identified.

KEY RESEARCH ACCOMPLISHMENTS:

- Identified ART-27 as a protein that interacts with the AR N-terminal subdomain spanning amino acids 153-336, including AF-1a (154-167) and a part of AF-1b (295-459)

- ART-27 enhances AR transcriptional activation when overexpressed in mammalian cells.

- Thus, ART-27 represents a novel AR N-terminus-associated coactivator.

REPORTABLE OUTCOMES:
Markus et al., Molecular Biology of the Cell 13: 670 (2002)

CONCLUSIONS:
We had identified ART27 as a novel AR N-terminal interacting protein, which plays a role in facilitating receptor-induced transcriptional activation. Validating ART27 as an AR regulatory protein in vivo is ongoing.

REFERENCES:

Amero, S. A., Kretsinger, R. H., Moncrief, N. D., Yamamoto, K. R., and Pearson, W. R. (1992). The origin of nuclear receptor proteins: a single precursor distinct from other transcription factors. Mol Endocrinol 6, 3-7.

Chou, P. Y., and Fasman, G. D. (1978). Empirical predictions of protein conformation. Annu Rev Biochem 47, 251-76.

Garnier, J., Osguthorpe, D. J., and Robson, B. (1978). Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. J Mol Biol 120, 97-120.

Hofmann, K., Bucher, P., Falquet, L., and Bairoch, A. (1999). The PROSITE database, its status in 1999. Nucleic Acids Res 27, 215-9.

Hong, H., Darimont, B. D., Ma, H., Yang, L., Yamamoto, K. R., and Stallcup, M. R. (1999). An additional region of coactivator GRIP1 required for interaction with the hormone-binding domains of a subset of nuclear receptors. J Biol Chem 274, 3496-502.
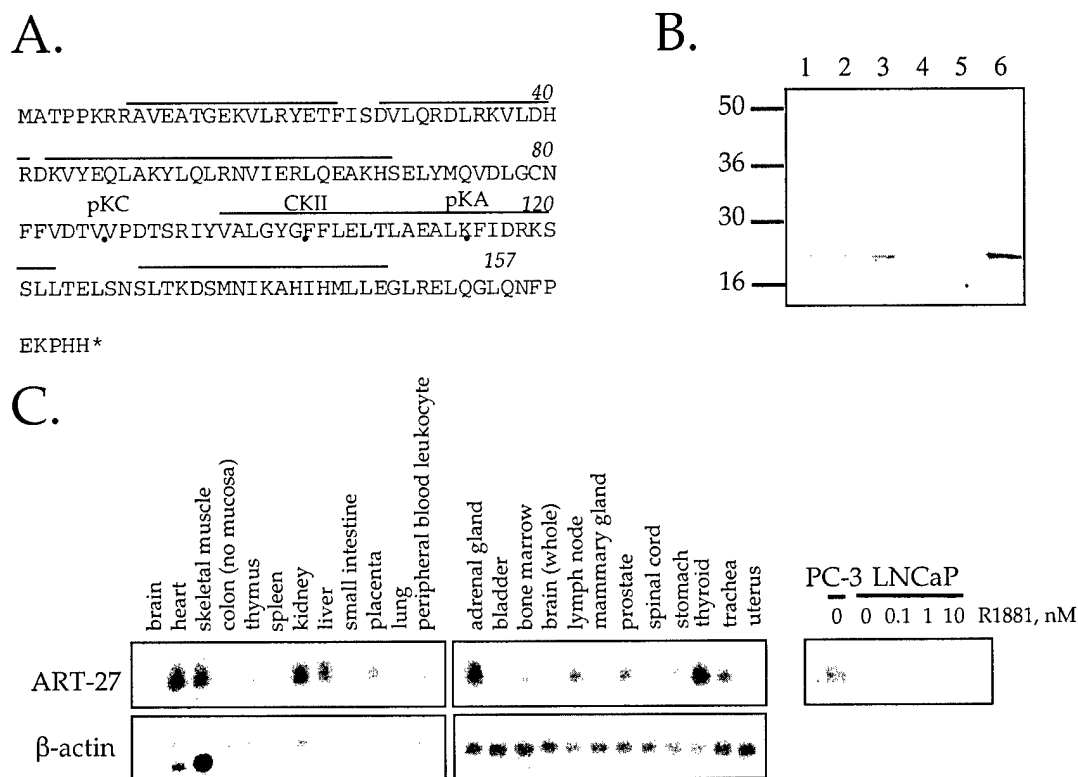
Owen, G. I., and Zelent, A. (2000). Origins and evolutionary diversification of the nuclear receptor superfamily. Cell Mol Life Sci 57, 809-27.

Saluja, D., Vassallo, M. F., and Tanese, N. (1998). Distinct subdomains of human TAFII130 are required for interactions with glutamine-rich transcriptional activators. Mol Cell Biol 18, 5734-43.

Schroer, A., Schneider, S., Ropers, H., and Nothwang, H. (1999). Cloning and characterization of UXT, a novel gene in human Xp11, which is widely and abundantly expressed in tumor tissue. Genomics 56, 340-3.

Shibata, H., Spencer, T. E., Onate, S. A., Jenster, G., Tsai, S. Y., Tsai, M. J., and O'Malley, B. W. (1997). Role of co-activators and co-repressors in the mechanism of steroid/thyroid receptor action. Recent Prog Horm Res 52, 141-64.

Tanese, N., Pugh, B. F., and Tjian, R. (1991). Coactivators for a proline-rich activator purified from the multisubunit human TFIID complex. Genes Dev 5, 2212-24.

# A.

```
                                                          40
MATPPKRRAVEATGEKVLRYETFISDVLQRDLRKVLDH

                                                          80
RDKVYEQLAKYLQLRNVIERLQEAKHSELYMQVDLGCN
      pKC              CKII            pKA    120
FFVDTVVPDTSRIYVALGYGFFLELTLAEALKFIDRKS

                                                          157
SLLTELSNSLTKDSMNIKAHIHMLLEGLRELQGLQNFP

EKPHH*
```

# B.



# C.



*Figure 1:* **Cloning and characterization of ART-27**

(A) Amino acid sequence of ART-27. The amino acid sequence of ART-27 is shown with an asterisk representing the stop codon. Potential phosphorylation sites for protein kinase C (pKC), casein kinase II (CKII), and protein kinase A (pKA) are marked by a dot below the target residue. Lines above the sequence represent predicted alpha-helical regions as determined by Chou-Fasman and Garnier-Osguthorpe-Robson algorithms. (B) Expression of ART-27 protein. Equal amounts (50 μg) of nuclear extracts prepared from HeLa cells (lanes 1), treated with TPA for 2 h (lane 2), serum-starved and stimulated with serum for 2 h (lane 3), PC-3 cells (lane 4) and whole cell extracts from COS1 cells transfected with pcDNA3 (lane 5) or pcDNA3:ART-27 (lanes 6) were analyzed by immunoblotting with affinity-purified anti-ART-27 antibody. No ART-27 immunoreactivity is observed with preimmune serum (our unpublished results). (C) ART-27 mRNA expression in human tissues and prostate cancer cell lines. A human multiple tissue Northern blot (Clontech: Human 12-lane MTN Blot I and II) containing 2 μg of poly A+ mRNA from the indicated tissues was hybridized with $^{32}$P-labeled probes corresponding to ART-27 (upper panel) and β-actin (lower panel). Total RNA was extracted from PC-3 and LNCaP cells cultured in the absence or presence of indicated doses of androgen (R1881) for 72 h. Equal amounts of RNA were separated on denaturing formaldehyde-agarose gels (see Materials and Methods), transferred to Duralon nylon membrane and hybridized to $^{32}$P-labeled cDNA probes corresponding to ART-27 (right panel). Equal loading for each lane is determined by ethidium bromide staining of the 28S rRNA (our unpublished results).

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| Regulatory Factors | ART-27 interaction strength |
|---|---|
| AR N-T | 10,000 |
| AR LBD | 1 |
| GR AF-1 | 14 |
| GR 30IIB | 8 |
| GR LBD | 1 |
| ER α | 19 |
| TAF130 | 3,374 |
| SRC-1 | 1 |
| CREB-N | 1 |
| Sp1A | 19 |
| Sp1B | 264 |
| VP16 | 1 |

*Figure 2:* **Specificity of AR:ART-27 interactions.**
Interaction of ART-27 with the AR N-terminus (N-T) (rat $AR_{18-500}$), AR LBD (rat $AR_{579-901}$) and other transcriptional regulatory factors was analyzed using the modified yeast two-hybrid assay. Regulatory factors examined include the GR AF-1 (rat $GR_{107-237}$), GR 30IIB (rat $GR_{107-237}$ E219K, F220L, W234R), SRC-1 ($SRC-1_{374-800}$), GR LBD (rat $GR_{525-795}$), $TAF_{II}130$ ($TAF_{II}130_{270-700}$), Sp1 A ($Sp1_{83-262}$), Sp1 B ($Sp1_{263-524}$), CREB-N ($CREB_{3-296}$), ERα ($ER\alpha_{1-595}$) and VP16. The strength of interaction is determined by a quantitative liquid β-galactosidase assay after a 24 h incubation in galactose media at 30°C and normalized to protein expression using the common HA-epitope resident on each protein. The background from the vector only control was subtracted from each sample. The interaction with ERα was observed in the presence, but not the absence (our unpublished results), of 100 nM 17β-estradiol. No interaction was observed with AR LBD and the GR LBD in either the presence or absence of 100 nM R1881 and 10 μM deoxycorticosterone, the preferred GR ligand in yeast (Garabedian and Yamamoto, 1992).

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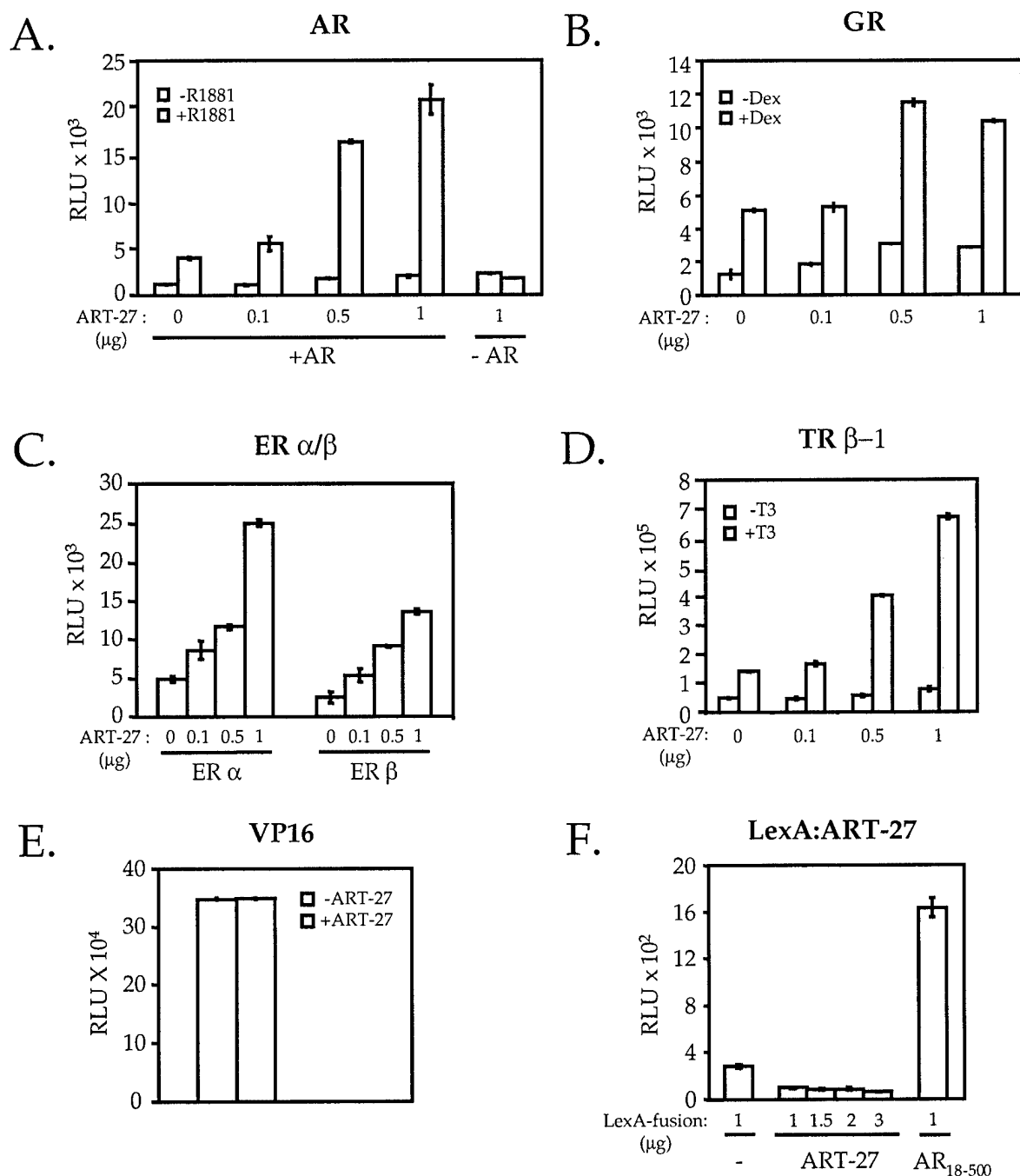
*Figure 3:* **ART-27 enhances steroid and thyroid receptor-dependent transcriptional activation.**
HeLa cells (1.2 x $10^5$ c ells/35 mm dish) were transiently transfected using Lipofectamine with paired receptor (0.2 μg/dish) and reporter constructs (0.1 μg/dish) for hAR + MMTV-Luc (A), GR + MMTV-Luc (B), ERα and ERβ + XETL (C), human TRβ-1 + pGL3-DR4 (D) along with the indicated amount of ART-27 or empty expression vector to equalize the total amount of DNA per dish and 0.05 μg pCMV-LacZ per dish as an internal control for transfection efficiency. Cells were treated with (A) 100 nM R1881, (B) 100 nM dexamethasone (Dex), (C) 10 nM 17β-estradiol (E2), (D) 100 nM triac (T3) (gray bars) or the ethanol vehicle (white bars) for 12 h and receptor transcriptional activation was assayed as described in Materials and Methods, normalized to β-galactosidase activity and expressed as relative luminescence units (RLU). The average of three independent experiments and standard error is shown. (E) ART-27 does not affect transactivation by VP16. HeLa cells were transfected as above with an expression construct for GAL4-VP16 and a reporter plasmid containing 5 Gal4 binding sites upstream of the E1b promoter, in the absence (white bars) or presence (gray bars) of 1 μg of ART-27 and assayed for luciferase activity. (F) ART-27 fails to activate transcription when tethered to DNA. HeLa cells were transfected as above with expression constructs for LexA, LexA-ART-27 or LexA-AR ₁₈₋₅₀₀. and the pΔ4X-LALO reporter plasmid and assayed for luciferase activity.

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## A. LexA-AR N-terminal derivative:
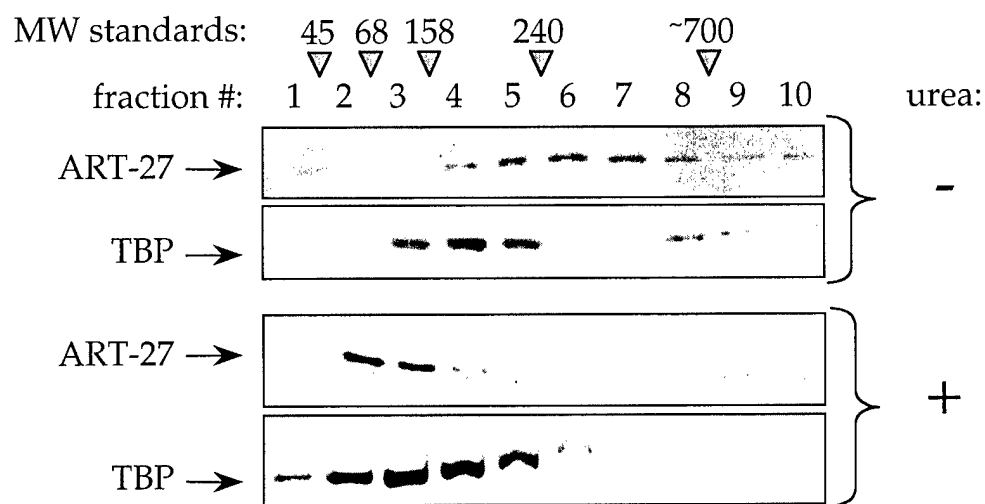


RLU x 10$^4$
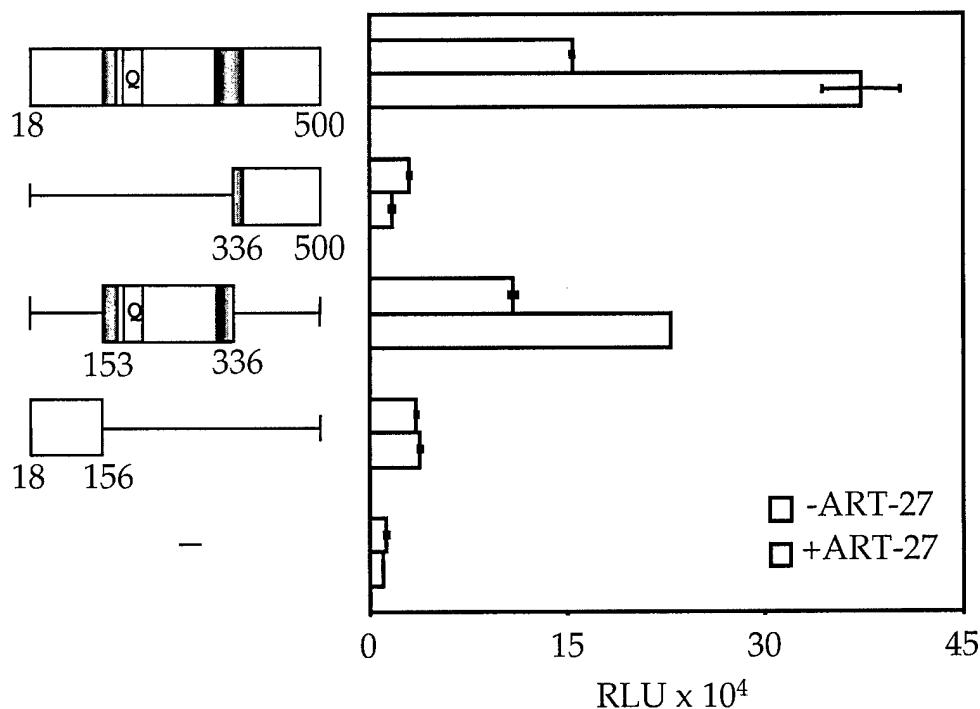
## B.
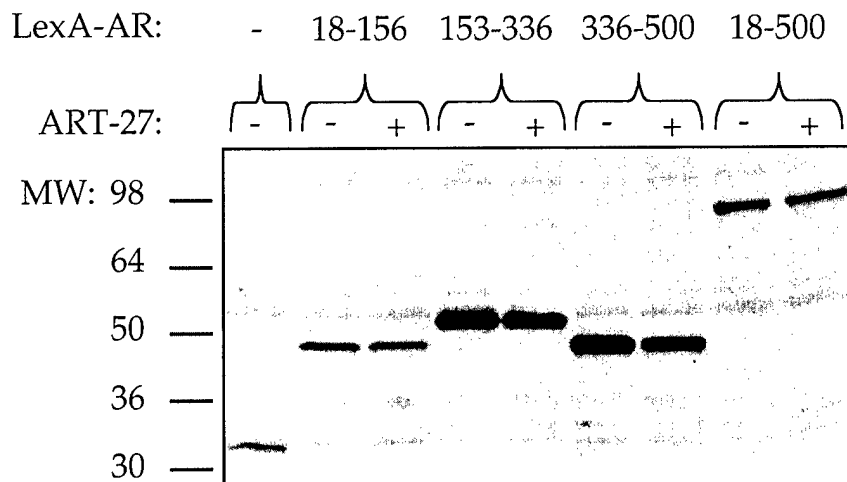


*Figure 4:* **The effect of ART-27 on AR transcriptional activation is dependent on the AR-interacting region.**
(A) HeLa cells were transfected with 0.5 μg of the pcDNA3LexA:AR N-terminal derivatives indicated and either 1.0 μg of the empty expression vector (white bars) or 1.0 μg pcDNA3-HA-ART-27 (gray bars) along with 1.0 μg of the LexA responsive-luciferase reporter (pΔ4X-LALO) and 0.25 μg pCMV-LacZ and AR activity was determined as described in Figure 5. Data represent the mean of duplicate data points normalized to β-galactosidase units. (B) The expression of the LexA:AR derivatives was analyzed by immunoblotting from a parallel set of transfections using a polyclonal antibody to LexA.

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MW standards:   45 68 158   240        ~700
                 ▽  ▽  ▽     ▽          ▽
     fraction #:  1  2  3  4  5  6  7  8  9  10        urea:

ART-27 →                                                     −

TBP →

ART-27 →                                                     +

TBP →

*Figure 5*: **Native ART-27 is part of a high molecular weight complex**
Nuclear extracts were prepared from HeLa cells and loaded onto linear 15% to 35% glycerol gradients with or without 2.4 M urea. Fractions were collected and analyzed by Western blot using affinity-purified ART-27 polyclonal or TBP monoclonal antibodies. Migration of molecular mass markers is indicated: 45 kDa and 68 kDa albumin; 158 kDa aldolase; 240 kDa catalase. 700 kDa is the molecular mass estimate of TFIID (Tanese et al., 1991).